Scientific Opinion on a notification (reference C/NL/09/01) for the placing on the market of the genetically modified carnation IFD-25958-3 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene

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Abstract: Genetically modified (GM) carnation IFD-25958-3 was developed to express anthocyanins in the petals conferring a mauve colour to the flowers. The GM carnation is intended to be imported in the European Union as cut flower for ornamental use only. Based on the molecular characterisation data, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) confirms the stability of the newly introduced trait and the absence of disruption of known endogenous genes. Since anthocyanins are common pigments in many food plants, it is not expected that accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods. Considering the ornamental use of cut flowers, and the limited exposure scenarios expected, the EFSA GMO Panel identified no reasons for any food safety concerns relating to carnation IFD-25958-3. The EFSA GMO Panel is also of the opinion that accidental release of GM carnations into the environment would not give rise to environmental safety concerns. The EFSA GMO Panel agrees with the methodology, including reporting intervals, proposed for post-market environmental monitoring. In response to the European Commission, the EFSA GMO Panel concludes that, in the light of the ornamental use of carnation IFD-25958-3 cut flowers, there is no scientific reason to consider that the placing on the market of the GM carnation will cause any adverse effects on human health or the environment.

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SCIENTIFIC OPINION

Scientific Opinion on a notification (reference C/NL/09/01) for the placing on the market of the genetically modified carnation IFD-25958-3 with a modified colour, for import of cut flowers for ornamental use, under Part C of Directive 2001/18/EC from Florigene[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

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ABSTRACT

Genetically modified (GM) carnation IFD-25958-3 was developed to express anthocyanins in the petals conferring a mauve colour to the flowers. The GM carnation is intended to be imported in the European Union as cut flower for ornamental use only. Based on the molecular characterisation data, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) confirms the stability of the newly introduced trait and the absence of disruption of known endogenous genes. Since anthocyanins are common pigments in many food plants, it is not expected that accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods. Considering the ornamental use of cut flowers, and the limited exposure scenarios expected, the EFSA GMO Panel identified no reasons for any food safety concerns relating to carnation IFD-25958-3. The EFSA GMO Panel is also of the opinion that accidental release of GM carnations into the environment would not give rise to environmental safety concerns. The EFSA GMO Panel agrees with the methodology, including reporting intervals, proposed for post-market environmental monitoring. In response to the European Commission, the EFSA GMO Panel concludes that, in the light of the ornamental use of carnation IFD-25958-3 cut flowers, there is no scientific reason to consider that the placing on the market of the GM carnation will cause any adverse effects on human health or the environment.

KEY WORDS

carnation, cut flower, delphinidin, *Dianthus caryophyllus*, Directive 2001/18/EC, import, petal colour

[1] On request from the European Commission, Question No EFSA-Q-2013-00328, adopted on 4 December 2014.

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SUMMARY

Following a request from the European Commission, the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on notification C/NL/09/01 from Florigene submitted under Part C of Directive 2001/18/EC[4]. The scope of notification C/NL/09/01 covers the import, distribution and retailing in the European Union (EU) of genetically modified (GM) carnation IFD-25958-3 cut flowers for ornamental use only.

In accordance with Directive 2001/18/EC, an assessment of the GM carnation was requested by the European Commission in order to address the outstanding objections raised by some Member States following the evaluation at the national level, and to assess the overall safety of the GM carnation. The EFSA GMO Panel was, therefore, asked to consider if there is any scientific reason to believe that the placing on the market of GM carnation IFD-25958-3 for import is likely to cause any adverse effects on human health or the environment.

In delivering the present scientific opinion, the EFSA GMO Panel considered the full notification C/NL/09/01, including additional information provided by the notifier, the assessment report of the Dutch competent authority, the concerns raised by Member States, relevant scientific publications and the experience gained in assessing GM carnations with similar traits (EFSA, 2006a, 2008; EFSA GMO Panel, 2014). The EFSA GMO Panel performed its risk assessment in accordance with the principles of its guidance documents on the risk assessment of GM plants for non-food or non-feed purposes (EFSA, 2009a) and on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010). It should be noted that the comparative compositional assessment as defined in the EFSA guidance documents (EFSA, 2006b; EFSA GMO Panel, 2011a) could not be applied to identify possible unintended effects of carnation IFD-25958-3.

The scientific evaluation by the EFSA GMO Panel included molecular characterisation of the inserted DNA and expression of the new proteins. A comparative evaluation of the morphological characteristics was undertaken, and the safety of the newly expressed proteins and of the whole GM plant was evaluated with respect to potential toxicity and allergenicity. The potential environmental impacts of accidental release of GM carnations into the environment and the post-market environmental monitoring (PMEM) plan proposed by the notifier were evaluated in the context of the scope of notification C/NL/09/01.

Carnation IFD-25958-3 has a modified flower colour, a shade of mauve, whereas the parental line has a cerise flower colour. The colour has been achieved by introducing into the parental carnation three expression cassettes which, together with other genes of the anthocyanin biosynthesis pathway that are already present in the non-GM carnation, give rise to the anthocyanins delphinidin and cyanidin, the same pigments that give colour to blueberry, blackcurrant and red grape. Carnation IFD-25958-3 is also tolerant to sulfonylurea herbicides, but this newly introduced trait was used only for the selection of transformed plants and not for plant protection purposes.

Carnation IFD-25958-3 was developed by *Agrobacterium tumefaciens*-mediated transformation of the conventional carnation Cerise Westpearl. The desired colour was obtained by introducing the flavonoid 3′,5′-hydroxylase (*f3′5′h*) coding sequence from *Viola* sp., the dihydroflavonol 4-reductase (*dfr*) coding sequence from *Petunia* sp. and a hairpin RNA interference (RNAi) construct which down-regulates endogenous *dfr*. Tolerance to sulfonylurea herbicides was conferred by the expression of a mutated *als* gene. The molecular characterisation data establish that carnation IFD-25958-3 contains a single insert consisting of the four expression cassettes. The stability of the newly introduced trait (mauve flower colour) was observed over multiple generations. Bioinformatic

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39.

analyses of the 5′ and 3′ flanking regions of the insert did not reveal disruption of known endogenous genes. Identities with allergens were found in putative translation products of open reading frames (ORFs) newly created by the genetic modification, but the likelihood that they are both transcribed and translated in carnation IFD-25958-3 was considered negligible.

Considering the scope of the notification and focusing on the limited information provided by the notifier, the EFSA GMO Panel is of the opinion that the altered flower colour and the differences observed for some morphological characteristics are not expected to influence the risk scenario of accidental intake of the GM carnation.

It is not expected that the accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods. Considering the scope of notification C/NL/09/01 and given that case reports of occupational allergies to carnations are rare, there are no indications that the genetic modification will increase the risk of allergy among those coming into contact with carnations. Considering the scope of notification C/NL/09/01 and the limited exposure scenarios expected, the EFSA GMO Panel identified no reasons for any safety concerns of carnation IFD-25958-3.

Carnation IFD-25958-3 cut flowers have marginal viability, negligible pollen production and few or no viable seeds. However, in the very unlikely event of escape into the environment via pollen/seeds or rooted plants, the EFSA GMO Panel considers that carnation IFD-25958-3 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, interactions with the biotic and abiotic environment are not considered to be relevant issues by the EFSA GMO Panel. The EFSA GMO Panel also concludes that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation IFD-25958-3 to environmental bacteria does not give rise to environmental safety concerns. The scope of the PMEM plan provided by the notifier is in line with the intended use of carnation IFD-25958-3. The EFSA GMO Panel agrees with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

In the light of the scope of notification C/NL/09/01 (import, distribution and retailing in the EU of carnation IFD-25958-3 cut flowers for ornamental use only), the EFSA GMO Panel concludes that there is no scientific reason to consider that the placing on the market of the GM carnation IFD-25958-3 will cause any adverse effects on human health or the environment.

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1. Introduction

Carnation IFD-25958-3 is a genetically modified (GM) variety of *Dianthus caryophyllus* L. used as a decorative plant species. The mauve colour of the flowers results from the expression of two newly introduced genes encoding flavonoid 3′,5′-hydroxylase (*f3′5′h*), dihydroflavonol 4-reductase (*dfr*) and a hairpin RNA interference (RNAi) gene, which down-regulates endogenous *dfr*. These three constructs, together with endogenous genes involved in the anthocyanin biosynthesis pathway, enable the biosynthesis of delphinidin in the petals. Carnation IFD-25958-3 also contains a mutated herbicide tolerance *als* gene coding for an acetolactate synthase (ALS) variant protein, used to facilitate the selection of GM shoots during the genetic transformation process.

In the present scientific opinion, the GM carnation IFD-25958-3 is evaluated by the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) in the light of the scope of notification C/NL/09/01 (import, distribution and retailing in the European Union (EU) of GM carnation cut flowers for ornamental use only).

Therefore, the EFSA GMO Panel evaluated the safety of carnation IFD-25958-3 for humans considering three possible routes of exposure through: (1) dermal contact, (2) inhalation and (3) accidental oral intake[5]. Owing to the scope of this notification, the EFSA GMO Panel did not assess the possible consequences of the intentional consumption of GM carnations by humans[6]. In relation to animals, both intentional and accidental oral intake were excluded from this opinion, as carnation IFD-25958-3 is not expected to enter the feed chain or to be accidentally consumed in the field (cultivation being excluded from the scope) (EFSA, 2009a).

Moreover, considering the scope of this notification, a very limited environmental exposure with respect to viable plant parts of the GM carnation is expected. Hence, the environmental risk assessment (ERA) is mainly concerned with the consequences of exposure through: (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives, (3) dispersal of seeds produced by GM cut flowers and possible progeny and (4) discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to recombinant DNA.

1.1. Background and Terms of Reference as provided by requestor

In July 2009, the European Commission received the full notification (reference C/NL/09/01), together with the positive assessment report from the competent authority of the lead Member State, The Netherlands.

In accordance with Directive 2001/18/EC[7], the notification was then transmitted to the competent authorities of other Member States. Some of them raised comments and objections during the statutory 60-day consultation period. The notifier, Florigene, provided the Member States with additional information in response to those comments and objections. However, some Member States maintained their reservations at the end of the additional 45-day consultation period, in which case, the European Commission is required to follow the procedure of Article 18(1) of Directive 2001/18/EC. In accordance with Article 18(1), the European Commission therefore consulted the EFSA GMO Panel for a scientific opinion.

Against this background, the EFSA GMO Panel identified mainly general comments rather than reasoned objections as in the sense of Directive 2001/18/EC. Moreover, concerns raised by Member

[5] Accidental oral intake should be considered as unintentional, infrequent and/or of relatively short duration.

[6] The EFSA GMO Panel is aware of a food habit in certain populations to intentionally consume carnation petals as garnish; however, this intentional use is outside the scope of this notification.

[7] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39.

States that relate, for example, to traceability, labelling, socio-economics, molecular detection methodologies and their validation fall outside the remit of EFSA or its GMO Panel.

EFSA is requested, in accordance with Article 28 of Directive 2001/18/EC, to provide a scientific opinion as to whether there is any scientific reason to believe that the placing on the market of the GM carnation line IFD-25958-3 for import is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

2. Data and methodologies

2.1. Data

The present evaluation of the safety of the GM carnation IFD-25958-3 by the EFSA GMO Panel is based on the information provided in notification C/NL/09/01, including additional information[8] provided by the notifier, the assessment report of the Dutch competent authority, the concerns raised by Member States, relevant scientific publications and the experience gained in assessing GM carnations with similar traits (EFSA, 2006a, 2008; EFSA GMO Panel, 2014).

2.2. Methodologies

The EFSA GMO Panel performed its risk assessment in accordance with the principles of its guidance documents on the risk assessment of GM plants for non-food or non-feed purposes (EFSA, 2009a) and on the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010).

3. Assessment

3.1. Molecular characterisation

3.1.1. Concerns raised by Member States

No objection raised by any Member State remained at the end of the 45-day Member States' consultation period. Therefore, notwithstanding its own risk analysis, the EFSA GMO Panel had no specific concerns to address from the Member States on the molecular characterisation of GM carnation IFD-25958-3.

3.1.2. Evaluation of relevant scientific data

3.1.2.1. Transformation process and vector constructs

To develop the IFD-25958-3 line, carnation variety Cerise Westpearl (CW) was transformed using disarmed *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*) strain AGL0, which carried the transformation vector pCGP3366.

The transformation vector pCGP3366 contained the following expression cassettes, which are needed to obtain the desired flower colour:

1. the dihydroflavonol 4-reductase (*dfr*) cassette, encompassing the promoter, the *dfr* coding sequence and the terminator, cloned as a whole from the *Petunia* × *hybrida*;
2. the flavonoid 3′,5′-hydroxylase (*f3′5′h*) cassette, containing the promoter sequence from *Antirrhinum majus* chalcone synthase (CHS) gene, the *f3′5′h* coding sequence from *Viola hortensis* derived from a complementary DNA (cDNA) clone and the terminator sequence of a gene encoding a *Petunia* × *hybrida* putative phospholipid transfer protein homologue;
3. the *dcdfr*hp cassette, containing the *Cauliflower mosaic virus* (*CaMV*) 35S promoter, a hairpin-forming construct targeted to specific, post-transcriptional down-regulation of

[8] See section 'Documentation provided to EFSA' below.

endogenous carnation *dfr* and the *CaMV* 35S terminator sequence. The hairpin-forming construct consisted of sense and antisense segments of the *dfr* coding sequence, separated by a petunia *dfr* intron.

These three cassettes were inserted into the plant genome to obtain the desired flower colour. In addition, the vector pCGP3366 contained the acetolactate synthase cassette (*als*), consisting of the *CaMV* 35S promoter and the coding region and the terminator sequence from a mutated *als* from the *SuRB* locus of *Nicotiana tabacum*. Acetolactate synthase provided tolerance to sulfonylurea herbicides and was used as a marker trait in the selection of transformants.

3.1.2.2. Transgene constructs in the genetically modified plants

Carnation IFD-25958-3 contains one insert consisting of the transfer DNA (T-DNA) region of the transformation vector pCGP3366. Southern blot and polymerase chain reaction (PCR) analyses indicated that no plasmid backbone sequences had been integrated into carnation IFD-25958-3. The sequences of the insert and the flanking regions were provided.

Bioinformatic analysis of the 5′ and 3′ flanking regions did not reveal disruption of known endogenous genes.

In order to assess if the open reading frames (ORFs) present within the insert and spanning the junction sites give rise to any safety issues, their putative translation products were compared to appropriate databases for similarities to known allergens and toxins by using suitable algorithms. There were no significant hits with known toxins. By using an 80-amino-acid sliding window approach, a 35 % sequence identity was found within the newly expressed protein ALS with a *Davidiella tassiana* protein (see section 3.3.2.2). By using the same approach, identity of over 35 % was found with allergen 'Amb a 4' from common ragweed *Ambrosia artemisiifolia* for ORF 5.242 within the insert. The putative translation product of ORF 5.242 is a 110-amino-acid sequence, generated from the reverse strand of the *als* gene cassette. Considering that ORF 5.242 is not in the codon frame intended to be expressed and does not include an ATG start codon, the likelihood that it is both transcribed and translated in carnation IFD-25958-3 is negligible. Furthermore, northern blot analysis did not reveal any other bands than the one expected for *als*. Searches of eight contiguous amino acids showed identity to a subtilisin protease allergen in *Bacillus licheniformis* for ORF 4.148 within the insert, putatively encoding a 25-amino-acid peptide. Considering that ORF 4.148 is not on the DNA strand of the insert intended to be transcribed and does not include an ATG, the likelihood that it is both transcribed and translated in carnation IFD-25958-3 is negligible.

3.1.2.3. Information on the expression of the insert

The presence of transcripts corresponding to *f3'5'h*, *dfr*, *dcdfr*hp and *als* genes in the petals was demonstrated using northern blot analysis. Confirmation of the functionality of *f3'5'h*, *dfr* and *dcdfr*hp was obtained visually from the flower colour, as well as from metabolite analysis using high-performance liquid chromatography (HPLC). Tolerance to sulfonylurea herbicides indicates the activity of the ALS protein.

3.1.2.4. Inheritance and stability of the inserted DNA

Carnation IFD-25958-3 was propagated vegetatively from April 2005 to September 2008, which represents multiple cycles of propagation. Furthermore, during 2007–2013, plants were cultivated in a field trial in Colombia and there were no incidents reported of flower colour change that would indicate genetic instability.

3.1.3. Conclusion

The molecular characterisation data establish that the carnation line IFD-25958-3 contains one insert, consisting of four expression cassettes responsible for the intended traits, i.e. mauve flower colour

conferred by the *dfr*, *dcdfr*hp and *f3'5'h* genes and herbicide tolerance conferred by the mutated *als* gene.

The results of bioinformatic analyses of the insert and flanking regions in carnation IFD-25958-3 did not indicate relevant similarities with known toxins. Identities with allergens were found in putative translation products of ORFs newly created by the genetic modification, but the likelihood that they are both transcribed and translated in carnation IFD-25958-3 is negligible. Sequence identity was found between the newly expressed protein ALS and an allergen from *Davidiella tassiana*. The safety relevance of this sequence identity is assessed in section 3.3.2.2.

3.2. Comparative analysis

3.2.1. Concerns raised by Member States

No objection remained among Member States concerning the comparative analysis of GM carnation IFD-25958-3 at the end of the 45-day Member States' consultation period.

3.2.2. Evaluation of relevant scientific data

Considering that there is no history of the use of carnations as food or feed to a significant degree, that the safety of carnations *per se* has never been assessed for food/feed uses and that the compositional profile of carnations is not known, the comparative compositional assessment as defined in the EFSA guidance documents (EFSA, 2006b; EFSA GMO Panel, 2011a) could not be fully applied to identify possible unintended effects in carnation IFD-25958-3 (see section 1 for further details).

3.2.2.1. Choice of comparator

Carnation IFD-25958-3 was compared with the non-GM carnation variety Cerise Westpearl (CW), which is the variety of carnation that was transformed to establish carnation IFD-25958-3. Carnation CW is characterised by cerise petals.

3.2.2.2. Compositional analysis

The contents of the anthocyanin colour pigments delphinidin, cyanidin and pelargonidin were determined in acetonitrile extracts of freeze-dried petals using HPLC in accordance with the method of Fukui et al. (2003). As these anthocyanins occur as glycosylated and/or acylated compounds in the plant, the analytical method used a hydrolysis step converting the pigments into their aglycones, allowing the determination of total delphinidin, total cyanidin and total pelargonidin and not the specific compounds in the plant.

The cerise flower petals of the comparator CW contained only cyanidin pigments (0.01 mg/g fresh weight (fw)) and pelargonidin pigments (1.06 mg/g fw), whereas those of the GM mauve-coloured carnation IFD-25958-3 contained delphinidin (0.54 mg/g fw), cyanidin (0.10 mg/g fw) and pelargonidin (0.02 mg/g fw). Delphinidin-based pigments were not observed in other plant tissues of the GM plants (stem, nodes, leaves and roots). This has been confirmed by HPLC studies of leaf and root material.

The altered levels and types of anthocyanins in carnation IFD-25958-3 account for the intended phenotypic changes in the flower colour. The altered flower colour is not expected to influence the risk scenario of accidental intake of the GM carnation.

3.2.2.3. Morphological traits and genetically modified phenotype

In total, 18 morphological characteristics were analysed in carnation IFD-25958-3 and its comparator (carnation CW) grown in a field trial in Australia, during the 2007–2008 season. An analysis of variance (ANOVA) identified eight significant differences between the GM carnation and its comparator. Thus, carnation IFD-25958-3 had a higher number of internodes per stem, a reduced length to the fifth node, a thinner stem at the fifth node, an increased calyx diameter and length, more

filaments, a reduced filament length and an increased number of petals per flower. In addition, the average number of days to flowering was shorter in carnation IFD-25958-3 than in carnation CW: 138 and 146 days, respectively. In response to a Member State comment, the notifier provided additional data from a field trial in Colombia. In that field trial, the average days to flowering and petal count per flower did not differ between carnation IFD-25958-3 and its comparator, whereas the other parameters that were statistically different in the Australian field trial were not investigated.

3.2.3. Conclusion

Considering the scope of the notification and focusing on the limited information provided by the notifier, the EFSA GMO Panel is of the opinion that the altered flower colour and the differences observed for some morphological characteristics are not expected to influence the risk scenario of accidental intake of the GM carnation. The relevance of the observed morphological differences for their potential environmental impacts is further assessed in section 3.4.3.1.

3.3. Food safety assessment

3.3.1. Concerns raised by Member States

No objection remained among Member States concerning the safety assessment of GM carnation IFD-25958-3 for humans at the end of the 45-day Member States' consultation period.

The possible need for an acute toxicity study with whole plant extracts to support the assessment of this GM carnation in relation to accidental intake by humans was suggested by a Member State and was also discussed by the EFSA GMO Panel. Such a study was not considered necessary, as the safety assessment is sufficiently supported by the available data (see sections 3.3.2.1(a) and (b)).

3.3.2. Evaluation of relevant scientific data

3.3.2.1. Toxicology

(a) Toxicological assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequences of the newly expressed proteins in carnation IFD-25958-3 reveal no significant similarities to known toxins to humans.

The EFSA GMO Panel has previously assessed the safety of the ALS, DFR and F3′5′H proteins and no reasons for concern were identified (EFSA, 2006a, 2008).

(b) Toxicological assessment of new constituents other than proteins

The anthocyanins delphinidin and cyanidin are present in carnation IFD-25958-3 at higher levels than in the non-GM carnation. These anthocyanins can also be found in many foods and, in some of them, at much higher concentrations than in the petals of carnation IFD-25958-3. Particularly high concentrations can be found, for example, in blackcurrants, aubergines, blueberries and red grapes (Cacho et al., 1992). According to Regulation 1333/2008[9] on food additives, anthocyanins (E 163) are authorised food additives in the EU. Anthocyanins have been evaluated by the Scientific Committee on Foods (SCF), which concluded that anthocyanins prepared by physical processes from natural foods are acceptable for use in food without further investigations. The SCF indicated that anthocyanins derived from natural sources are only acceptable as food additives if the quantities ingested do not differ substantially from the amounts that are likely to be ingested as a result of the normal consumption of the foods in which they occur naturally (SCF, 1975). In the re-evaluation of anthocyanins, the Scientific Panel on Food Additives and Nutrient Sources Added to Food of EFSA (EFSA ANS Panel, 2013) concluded that, provided that exposure from the use of food colours is

[9] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

comparable to that from the diet, the conclusion on safety in the 1975 opinion would still apply to anthocyanins extracted by aqueous processes from edible fruits and vegetables.

It is not expected that the accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods. Therefore, the EFSA GMO Panel sees no reason for concern regarding the presence of delphinidin and cyanidin in petals of carnation IFD-25958-3.

In addition, the notifier also performed a study on gene mutations in bacteria using *Salmonella enterica* Typhimurium (Ames test) with water extracts of leaves or petals of carnation IFD-25958-3 and the control carnation CW. The water extracts did not show mutagenic activity under the conditions of the assay.

(c) Toxicological assessment of the whole genetically modified plant

Given that carnation IFD-25958-3 is not intended for human consumption as food but is intended for ornamental use only, the EFSA GMO Panel considered the possible effects of the genetic modification on human health in the case of accidental intake (EFSA, 2009a). Considering the assessment of the newly expressed proteins (section 3.3.2.1(a)) and of the new constituents other than proteins (section 3.3.2.1(b)), the EFSA GMO Panel identified no reasons for concern.

3.3.2.2. Allergenicity

(a) Allergenicity assessment of newly expressed proteins

Bioinformatic analyses of the amino acid sequence of the newly expressed proteins in carnation IFD-25958-3 using the criterion of more than 35 % identity in a segment of 80 or more amino acids (Codex Alimentarius, 2003) revealed no significant similarities to known allergens. In addition, the notifier performed analyses searching for matches of eight contiguous identical amino acid sequences between these newly expressed proteins and known allergens, which confirmed the outcome of the above-mentioned bioinformatic analyses showing no similarities to known allergens.

The EFSA GMO Panel has previously assessed the safety of the ALS, DFR and F3′5′H proteins and no reasons for concern were identified (EFSA, 2006a, 2008).

The EFSA GMO Panel noted that 35 % sequence identity over a window of 80 amino acids between the ALS protein and an allergen from *Davidiella tassiana* was reported. However, this hit was not considered relevant because the percentage of identity was not greater than 35 % and, in addition, a high E-value[10] was seen.

(b) Allergenicity assessment of the whole genetically modified plant

Occupational allergy (dermal and respiratory allergies) to carnations in workers handling cut flowers/carnations over a long time has been described (Sanchez-Guerrero et al., 1999; Cistero-Bahima et al., 2000; Sanchez-Fernandez et al., 2004; Stefanaki and Pitsios, 2008)[11]. This allergy could be caused by the flower, by mites such as *Tetranychus urticae* infesting carnations or by both simultaneously. Nevertheless, case reports of occupational allergies to carnations are rare.

More recently, a case report of an individual with a respiratory allergy to carnations with no occupational exposure was published (Brinia et al., 2013)[11].

[10] An alignment derived from a FASTA search of a database is accompanied with an E-value, which represents the number of times the corresponding alignment score is expected at chance.
[11] Additional information 28 August 2014.

According to the notifier, no adverse reactions (including allergenicity or contact dermatitis) to GM carnation IFD-25958-3 cut flowers used for ornamental purposes have been reported in the populations handling the flowers (growers, distributors and purchasers) where it is produced and/or marketed[11].

Considering the scope of notification C/NL/09/01 and given that case reports of occupational allergies to carnations are rare, there are no indications that the genetic modification will increase the risk of allergy among those coming into contact with carnations.

3.3.3. Conclusion

Carnation flowers have a long history of use as ornamentals. Carnation IFD-25958-3 differs from the non-GM carnation in that it synthesises anthocyanins, mainly delphinidin and cyanidin, in the petals, which confer a mauve colour to the flowers. Delphinidin and cyanidin are common pigments in many ornamental flowers and food plants such as red grapes, blackcurrants, aubergines and blueberries. It is not expected that accidental intake of petals of carnation IFD-25958-3 would contribute substantially to the overall intake of anthocyanins from foods.

Considering the scope of notification C/NL/09/01 and given that case reports of occupational allergies to carnations are rare, there are no indications that the genetic modification will increase the risk of allergy among those coming into contact with carnations.

Considering the scope of notification C/NL/09/01 and the limited exposure scenarios expected, the EFSA GMO Panel identified no reasons for any safety concerns of carnation IFD-25958-3.

3.4. Environmental risk assessment and post-market environmental monitoring plan

3.4.1. Concerns raised by Member States

Some Member States expressed concerns related to the possible illegal propagation of carnation IFD-25958-3 through vegetative multiplication by individuals. More details on the methods and implementation of the post-market environmental monitoring (PMEM) plan were requested by some Member States.

3.4.2. Evaluation of relevant scientific data

Considering the scope of notification C/NL/09/01, there will be very limited environmental exposure with respect to viable plant parts of the GM carnation. The ERA is mainly concerned with the consequences of exposure through: (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives, (3) dispersal of seeds produced by GM cut flowers and possible progeny and (4) discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to recombinant DNA.

3.4.3. Environmental risk assessment[12]

3.4.3.1. Potential unintended effects on plant fitness due to the genetic modification

Carnation is the common name of *Dianthus caryophyllus* (i.e. cultivated carnation). Members of the genus *Dianthus*, including wild and domesticated species, are fairly diverse, as their origins range from southern Russia to the Alpine region of Greece and the Auvergne mountains of France. *Dianthus* spp. are adapted to the cooler Alpine regions of Europe and Asia, and are also found in Mediterranean coastal regions. *D. caryophyllus* is a widely cultivated ornamental plant in Europe both in glasshouses and outdoors (i.e. in Italy and Spain) and is occasionally naturalised in some Mediterranean countries

[12] Notification C/NL/09/01, Section B.

but appears to be restricted to the coastal Mediterranean regions of Greece, Italy, Sicily, Corsica and Sardinia (Tutin et al., 1993).

Although *Dianthus* spp. do not spread vegetatively through organs such as bulbs, stolons or rhizomes, the cultivated carnations can be vegetatively propagated to produce plants for cut flower production. Cuttings are taken from 'mother plants/stems' which are continually pruned to produce a large number of vegetative cuttings from axillary buds. These cuttings are rooted in conditions of high humidity after treatment to encourage root growth. Rooted plants may be planted in soil or grown hydroponically, and are kept for one to two years. Flowers are produced in flushes, beginning three to five months after rooted cuttings are planted.

The majority of *Dianthus* spp. is self-sterile because the stigma is not receptive to pollen until one week or more after anthers have shed pollen. Cultivated carnations require pollination by hand to set seed (Bird, 1994). As a result of the long history of use of vegetative propagation and selection for flower characteristics, the carnation produces only a negligible amount of pollen, and consequently seed set is low or absent (Galbally and Galbally, 1997). The quantity and quality of pollen varies with the cultivar (Kho and Baer, 1973; Galbally and Galbally, 1997). Carnation pollen is heavy and sticky and has low viability. Wind plays little role in pollen dispersal (OGTR, 2006). In the wild, cross-pollination of *Dianthus* spp. is by insect pollinators, in particular by Lepidoptera, which have probosces of sufficient length to reach the nectaries at the base of the flowers.

Carnation IFD-25958-3 has a modified flower colour resulting from the expression of two newly introduced genes encoding *f3'5'h*, *dfr* and a hairpin RNAi gene, which down-regulates endogenous *dfr*. These three constructs, together with endogenous genes involved in the anthocyanin biosynthesis pathway, enable the biosynthesis of delphinidin in the petals. These anthocyanins are also widely found, for example, in flowers of the genus *Petunia* (Ando et al., 1999), *Rosa* (Biolley and Jay, 1993) or *Chrysanthemum* (Schwinn et al., 1993; Andersen et al., 2000). There is no evidence that the presence of delphinidin and cyanidin would lead to effects on plant fitness.

Carnation IFD-25958-3 contains a mutated *als* gene conferring tolerance to sulfonylurea herbicides. Given that the ALS enzyme is needed for the biosynthesis of some branched-chain amino acids such as isoleucine, ALS-inhibiting herbicides cause the death of the plant by interfering with this biosynthesis pathway. In relation to this, Tranel and Wright (2002) reported that tolerance to ALS-inhibiting herbicides was widespread among weeds and was mostly due to a mutated *als* gene. In addition, the ALS-tolerant biotype was shown to be less sensitive to feedback inhibition by branched-chain amino acids. This results in greater accumulation of branched-chain amino acids in tolerant biotypes, which may allow seeds from tolerant biotypes to germinate more rapidly, especially in cool temperatures. This may indicate a possible change in behaviour of the tolerant plants in the absence of herbicide selection in the very unlikely event of escape into the environment. Wild *Dianthus* populations exhibit a diversity of phenotypes exploiting niches in a wide geographical range in Europe (Tutin et al., 1993). The EFSA GMO Panel considered that a small change in seed germination characteristics induced by ALS tolerance is unlikely to be outside the current range of seed germination characteristics currently expressed by non-GM carnations and thus is unlikely to have an ecological impact.

In the very unlikely event of gene transfer to cultivated carnations, they may express the mutated *als* gene conferring tolerance to sulfonylurea herbicides. This could result in a possible fitness advantage and higher weediness of the tolerant plants in the presence of these herbicides and those with a similar mode of action. However, these herbicides are not known to be used[13] on cultivated carnations. Such herbicide-tolerant plants can be managed by a range of measures (Tranel and Wright, 2002). There is no evidence that the traits introduced by the genetic modification would result in increased persistence and invasiveness of a plant species.

[13] See additional information provided by the notifier to Member States after the 60-day consultation period.

In general, carnation varieties compete poorly outside their cultivated environment. In addition, carnation varieties do not show weedy characteristics. In notification C/NL/09/01, the notifier presented morphological or phenotypic data gathered from a field trial conducted in Australia during the 2007–2008 season (see section 3.2.2.3)[14]. A total[15] of 18 morphological characteristics were evaluated for the GM carnation, in comparison with the parental line CW. Statistically significant differences were observed between the GM carnation and its parental line for 8 out of the 18 characteristics studied. Carnation IFD-25958-3 had a higher number of internodes per stem, a reduced length to the fifth node, a thinner stem at the fifth node, an increased calyx diameter and length, more filaments, a reduced filament length and an increased number of petals per flower. The notifier attributed these variations in morphological characters to environmental factors. The notifier also reported from the 2007–2008 Australian field trial a lower average number of days to flowering for the GM carnation than its parental line. However, the notifier did not observe any difference in the number of petals per flower and mean days to flowering between the GM and non-GM carnation from a supplementary field trial carried out in Colombia. The EFSA GMO Panel is of the opinion that, owing to their morphological nature, these characteristics for which differences were observed are unlikely to affect the survival, establishment and fitness of the GM carnation.

Moreover, the EFSA GMO Panel is not aware of any scientific reports of increased spread and establishment of (GM) carnations or of any change in survival capacity, including overwintering (COGEM report[16]; EFSA, 2006a, 2008). In addition, *D. caryophyllus* has been imported into all EU countries as a garden ornamental plant and cut flower for many decades and EFSA is not aware of any reports of feral populations that have established outside of cultivation.

In order to assess the likelihood of unintended effects on the environment, the EFSA GMO Panel followed a weight-of-evidence approach in collating and assessing appropriate information from various data sources (e.g. molecular data, available phenotypic data from field trials performed by the notifier, literature and previous evaluations of similar transformation events (EFSA, 2006a, 2008; EFSA GMO Panel, 2014)). Therefore, considering the scope of notification C/NL/09/01 and the data available, the EFSA GMO Panel considered that there would be very little exposure of other *Dianthus* populations and no changes in plant characteristics of any ecological significance. Carnation IFD-25958-3 plants would show changed fitness characteristics only when exposed to sulfonylurea herbicides, but these herbicides are not used in carnation cultivation or in habitats where wild *Dianthus* spp. might occur. The EFSA GMO Panel also concludes that the propagation of the GM carnation (e.g. rooting) by individuals cannot be excluded. However, should this occur, carnation IFD-25958-3 would not show any potential for increased survival, fitness or weediness compared with its parental line.

3.4.3.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, through either horizontal gene transfer of DNA or vertical gene flow via seed dispersal and cross-pollination.

(a) Plant-to-bacteria gene transfer

Considering the scope of notification C/NL/09/01, the ERA is concerned with exposure through discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to

[14] Notification C/NL/09/01, Attachment A12.

[15] Plant height (mm), number of internodes per stem, length of fifth node (mm), thickness of fifth node (mm), leaf length of third node from the top (mm), height of corolla (mm), flower diameter (mm), calyx diameter (mm), calyx length (mm), number of lobes per calyx, number of petals per flower, petal length (mm), petal width (mm), number of styles, style length (mm), number of viable anthers, filament length (mm), number of filaments.

[16] Available online: http://www.cogem.net/index.cfm/en/publications/publicatie/advisory-report-import-distribution-and-retail-of-cut-flowers-with-modified-flower-colour-gm-carnation-shd-27531-4

recombinant DNA. If accidental intake of these GM carnations by humans occurs[17] (see section 3.3), it is likely to be at very low levels so that exposure of gastro-intestinal tract bacteria and microfloral decomposers of faecal material will be very low.

Current scientific knowledge of recombination processes in bacteria indicates that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as plants to microorganisms) is not likely to occur at detectable frequencies under natural conditions (see EFSA, 2009b, for further details).

Successful horizontal gene transfer would require the stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred on the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. This requires the presence of stretches of DNA sequences that are similar in the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions have sequence similarity with bacterial sequences in the recipient.

Carnation IFD-25958-3 does not contain genetic elements with identity or high similarity to those of bacteria. The recombinant genetic elements used for the construction of carnation IFD-25958-3 originate from plants, i.e. *Petunia*, *Viola*, *Antirrhinum* and *Nicotiana tabacum* (tobacco) (for more details, see section 3.1.2.1). Owing to the absence of DNA with high similarity to that of bacteria, there is no indication of facilitated transfer of recombinant genes to bacteria when it is compared with the transfer of genes from non-GM carnations. Thus, based on the data provided by the notifier, no increased likelihood of horizontal gene transfer from carnation IFD-25958-3 to environmental bacteria is expected. The EFSA GMO Panel could not identify any selective advantage which would be provided to environmental bacteria when receiving the recombinant DNA of carnation IFD-25958-3.

Therefore, considering the scope of notification C/NL/09/01, the EFSA GMO Panel concluded that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation IFD-25958-3 to environmental bacteria does not give rise to environmental safety concerns.

(b) Plant-to-plant gene transfer

Considering the scope of notification C/NL/09/01, the ERA is mainly concerned with indirect exposure through (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives and (3) dispersal of seeds produced by GM cut flowers and possible progeny.

Carnation IFD-25958-3 plants are imported as cut flowers and thus have no roots and only occasional vegetative buds. The cut stems with vegetative shoots could be propagated by rooting or by micro-propagation. The latter is a multiplication technique applied in the laboratory which requires particular expertise and adequate material for successful tissue culture. The EFSA GMO Panel is of the opinion that this technique is unlikely to be used by individuals (e.g. amateur gardeners) to propagate GM carnations. However, the GM carnation could be propagated by rooting and then released into the environment (e.g. gardens). The EFSA GMO Panel therefore considered the consequences of such potential releases (see section 3.4.3.1) and concluded that, should this occur, carnation IFD-25958-3 would not show any potential for increased survival, fitness or weediness compared with its parental line.

Wind plays little role in pollen dispersal of *Dianthus* spp. (OGTR, 2006). In the wild, cross-pollination of *Dianthus* spp. is by insect pollinators, in particular by Lepidoptera, which have probosces of sufficient length to reach the nectaries at the base of the flowers. However, the GM carnation has double flowers with a high density of petals. These obstruct insect pollinators from probing the

[17] Accidental oral intake should be considered as unintentional, infrequent and/or of relatively short duration.

flowers to reach the nectaries and therefore discourage insect pollinator activity and limit the amount of pollen they collect and transfer to other flowers. The EFSA GMO Panel is of the opinion that the potential spread of pollen of the GM carnation by Lepidoptera to wild *Dianthus* spp. cannot be eliminated but is highly unlikely to occur and, if it did occur, it is very unlikely that viable hybrids would be produced, survive and cause adverse environmental effects (see section 3.4.3.1).

Moreover, the reproductive biology of *Dianthus* (OGTR, 2006) and the information provided by the notifier suggest that pollen production by flowers and pollen viability are low. Moreover, in notification C/NL/09/01, the notifier pointed out that '*flowers must be harvested in tight bud (or closed bud for spray types) for distribution and marketing*', which rules out pollination during production, but suggests it could occur post-marketing. However, the data indicate that pollen transfer to other carnations is very unlikely to occur owing to very low fertility levels in most carnations. In addition, viable seed production of cut flowers is very unlikely and has not been observed to date with carnation IFD-25958-3, most probably because of its limited life time (i.e. three weeks) in comparison with the time needed for complete seed development (i.e. five weeks).

The EFSA GMO Panel also considered the possibility of natural exchange of genetic material with other carnation varieties, *Dianthus caryophyllus* L., and wild *Dianthus* species. Although hybridisation is mentioned in some floristic surveys, the EFSA GMO Panel is not aware of reports of gene flow between cultivated carnations and wild *Dianthus* spp. in the literature. The probability of spontaneous hybridisation between the GM carnation and other cultivated carnations or wild relatives, and then the establishment of viable hybrids, is considered to be very low.

Therefore, taking account of the very low potentials for hybridisation and/or seed production of (GM) carnations, the EFSA GMO Panel concludes that plant-to-plant gene transfer of the introduced genes is very unlikely and, if it did occur, it is unlikely to result in viable seed production leading to adverse environmental effects.

3.4.3.3. Potential interactions of the genetically modified plant with target organisms

Considering the scope of notification C/NL/09/01 and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered a relevant issue by the EFSA GMO Panel.

3.4.3.4. Potential interactions of the genetically modified plant with non-target organisms

Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered a relevant issue by the EFSA GMO Panel.

3.4.3.5. Potential interactions with the abiotic environment and biogeochemical cycles

Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered a relevant issue by the EFSA GMO Panel.

3.4.4. Post-market environmental monitoring[18]

According to Annex VII of Directive 2001/18/EC, the objectives of a post-market environmental monitoring (PMEM) plan are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO or its use on human health or the environment that were not anticipated in the ERA.

[18] See notification C/NL/09/01, Section D.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the PMEM plan provided by the notifier (EFSA GMO Panel, 2011b). The potential exposure to the environment of carnation IFD-25958-3 would be mainly through (1) unintended release into the environment of GM carnations obtained by vegetative multiplication, (2) pollen dispersal from GM cut flowers to other carnations and wild relatives, (3) dispersal of seeds produced by GM cut flowers and possible progeny and (4) discarded GM carnation cut flowers resulting in possible exposure of environmental bacteria to recombinant DNA. The scope of the PMEM plan provided by the notifier is in line with the restricted intended use of GM carnation cut flowers.

The PMEM plan proposed by the notifier includes (1) a questionnaire for the European importers and operators, including questions on unexpected adverse effects and '*illegal growing*'; (2) the involvement of existing networks (i.e. national plant protection services); and (3) the consultation of a network of European taxonomists, botanists and breeders to report on any wild populations or unusual *Dianthus* hybrids that might originate from the GM carnation. In addition, the notifier plans to survey the production sites in Colombia and Ecuador to report diverse observations, including adverse effects and the incidence of genetic off-types. The notifier proposes to submit a PMEM report on an annual basis. The report will include, for example, the number of imported GM cut flowers and a report of the identified hybrids and of feral carnation populations, if any.

The EFSA GMO Panel is of the opinion that the scope of the PMEM plan proposed by the notifier is in line with the restricted intended use of carnation IFD-25958-3, as the ERA did not cover cultivation and identified no potential adverse environmental effects. No case-specific monitoring is required.

3.4.5. Conclusion

Carnation IFD-25958-3 cut flowers have marginal viability, negligible pollen production and few or no viable seeds. However, in the very unlikely event of escape into the environment via pollen/seeds or rooted plants, the EFSA GMO Panel considers that carnation IFD-25958-3 would not show enhanced fitness characteristics, except when exposed to sulfonylurea herbicides. Considering the scope of notification C/NL/09/01 and the low level of exposure to the environment, interactions with the biotic and abiotic environment are not considered to be relevant issues by the EFSA GMO Panel. The EFSA GMO Panel also concluded that the unlikely, but theoretically possible, horizontal gene transfer of recombinant genes from carnation IFD-25958-3 to environmental bacteria does not give rise to environmental safety concerns. The scope of the PMEM plan provided by the notifier is in line with the intended use of carnation IFD-25958-3. The EFSA GMO Panel agreed with the general methods and approaches, including reporting intervals, proposed by the notifier in its PMEM plan.

4. Conclusions

In the light of the scope of notification C/NL/09/01 (import, distribution and retailing in the EU of carnation IFD-25958-3 cut flowers for ornamental use only), the EFSA GMO Panel concludes that there is no scientific reason to consider that the placing on the market of the GM carnation IFD-25958-3 will cause any adverse effects on human health or the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Notification C/NL/09/01 under Part C of Directive 2001/18/EC submitted by Florigene to the European Commission, and received from the European Commission on 4 April 2013.

2. Letter from the European Commission, dated 4 April 2013, to the EFSA Executive Director concerning a request for the placing on the market of genetically modified carnation IFD-25958-3 under Directive 2001/18/EC by Florigene.

3. Acknowledgement letter, dated 7 June 2013, from EFSA to the European Commission.

4. Letter from EFSA to the notifier, dated 24 July 2013, requesting additional information.

5. Letter from the notifier to EFSA, received on 8 November 2013, providing additional information.
6. Letter from EFSA to the notifier, dated 19 December 2013, requesting additional information.
7. Letter from the notifier to EFSA, received on 23 December 2013, providing additional information.
8. Letter from EFSA to the notifier, dated 3 April 2014, requesting additional information.
9. Letter from the notifier to EFSA, received on 25 April 2014, providing additional information.
10. Letter from EFSA to the notifier, dated 29 July 2014, requesting additional information.
11. Letter from the notifier to EFSA, received on 26 August 2014, providing additional information.
12. Letter from EFSA to the notifier, dated 2 October 2014, requesting additional information.
13. Letter from the notifier to EFSA, received on 29 October 2014, providing additional information.

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